

# Dynamics of Macro- and Micronutrients in Cuphea

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## ABSTRACT

Temporal dynamics of nutrient densities, their interrelationships, and remobilization from leaves to seeds of cuphea were quantified in growth chamber and field studies. Temporal nutrient densities in leaf samples exhibited large levels of variation, whether remobilized and largely accumulated in the seed [copper, (Cu), potassium (K), phosphorus (P), sulfur (S) and zinc (Zn)], remobilized and accumulated in the seed coat [boron (B), calcium (Ca), iron (Fe), magnesium (Mg), manganese (Mn), and sodium (Na)] or almost excluded from the seed [barium (Ba), selenium (Se) and strontium (Sr)]. The temporal seed-to-leaf nutrient density “[S]/[L]” ratios and the proportion of variance unique to each nutrient separated the nutrients into a group (Cu, Fe, S, and Zn) with large [S]/[L] ratios and large unique variances, and another group (B, Ca, Mg, Mn and Sr) with small [S]/[L] ratios and small unique variances; the first group was selectively stored in the developing embryo. Nutrients with large densities in leaves at harvest may constitute a resource potentially available for subsequent crops.

**Keywords:** cuphea, oil crop, nutrient density, nutrient homeostasis

## INTRODUCTION

Understanding the homeostatic mechanisms that delineate nutrient accumulation and remobilization in the cuphea germplasm line PSR23, their dynamics, and interrelationships would help toward its development as a competitive oilseed crop. Olness et al. (2004) speculated that poor early seedling growth and stand establishment of PSR23, a germplasm line derived from an inter-specific *Cuphea* spp. Cross (*C. lanceolata* and *C. viscosissima*) with partial

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seed retention (Knapp and Crane, 2000), are caused by an inadequately developed root system and low availability of certain nutrients, especially phosphorus (P). Seed reserves of macro- and micronutrients are essential for the first phase of seedling development; therefore, nutrients in cuphea seed may constitute a significant source of several essential elements necessary to enhance its germination and early seedling growth. Phenotypic diversity in seed quality traits such as nutrient content could be crucial for genetic improvement and establishment of potential oilseed crops (Rahamatalla et al., 1998; Dierig et al., 2003). An overview of variation within the available cuphea germplasm can be utilized in breeding approaches, specifically to develop populations that combine balanced nutrient content, larger seed size, and larger oil content.

Significant genotypic differences in the density of macro- and micronutrients have been exploited for the improvement of corn, *Zea mays* (Feil et al., 2005); soybean, *Glycine max* (Naevé and Shibbes, 2005); field bean, *Phaseolus vulgaris* (Moraghan and Grafton, 2001); and wheat, *Triticum aestivum* (Anglani, 1998). Moreover, species differ in the uptake of selected nutrients by temperate grasses and legumes have been used to combine desirable agronomic traits and nutrient uptake capacity (Brink et al., 2001). Nitrogen (N), P, potassium (K), and sulfur (S) relationships have been found to impact yield and oil content of canola, *Brassica napus* L. var *napus*, which retained about 60% of the N, 70% of the P, but only 15% of the K and S in its seed, while the remaining portions remained in the postharvest residue (Jackson, 2000). The genetic basis of nutrient associations in *Silene vulgaris* (Ernst et al., 2000), *Arabidopsis thaliana* (Vreugdenhil et al., 2004), and in common bean *Phaseolus vulgaris* (Moraghan and Grafton, 2001) have been determined and attributed to either pleiotropic effects, due to physiological coupling of the accumulation of certain nutrients, or to linkages among different genes. No information is available on densities of macro- and micronutrients in cuphea, and on their dynamics and interrelationships during the growing season. Therefore, it is imperative to gain insight into the magnitude of variability present in a cuphea germplasm line (PSR23) for macro- and micronutrients and their dynamics, as this will provide the basis for effective selection and potential improvement of biological and oil yields of this potential oilseed crop (Olness et al., 2004). The objective of this study was to quantify the temporal dynamics and interrelationships of macro- and micronutrients in leaves and seeds of the cuphea germplasm line PSR23 (referred to hereafter as cuphea) grown to maturity in a growth chamber and field studies.

## MATERIALS AND METHODS

### Growth Chamber Study

An EGC Model M54 growth chamber (Environmental Growth Chambers, Chagrin Falls, Ohio, USA) was used to grow cuphea plants from germination to full

seed maturity. The chamber was programmed to simulate natural diurnal temperature and light conditions. The light/dark temperatures and light conditions were set at 22/20°C and 16/8 hours, respectively. The chamber program gradually ramps both temperature and photoperiod starting at 0600 and terminating at 2200 CST (Central Standard Time). Photosynthetic active radiation inside the growth chamber was maintained at  $\sim 700 \mu\text{mol m}^{-2}\text{s}^{-1}$  using incandescent and Very High Output (VHO) fluorescent bulbs. Environmental conditions, namely temperature and humidity, were logged continuously throughout the study. Cuphea seeds were germinated under controlled conditions before single plants were transplanted individually to 11.4 L plastic pots filled with a 40:40:20 mixture of soil (Barnes, fine loamy, mixed superactive, frigid Calcic Hapludoll; see below), sand, and peat. A fertilizer solution was freshly prepared every week and was composed of nitrogen (N; 20%), phosphorus (as  $\text{P}_2\text{O}_5$ ; 19%), potassium (as  $\text{K}_2\text{O}$ ; 18%), magnesium (Mg; 0.15%), copper (Cu; 0.01%), iron (Fe; 0.1%), manganese (Mn; 0.056%), and zinc (Zn; 0.0162%). These nutrients were derived, respectively, from ammonium phosphate, potassium nitrate, magnesium sulfate, copper ethylenediaminetetraacetic acid (EDTA), iron diethylenetriaminepentaacetic acid (DTPA), manganese EDTA, and zinc EDTA. The fertilizer solution was prepared by mixing 1 g dry fertilizer  $\text{L}^{-1}$  of deionized water. Plants were fertilized weekly with 0.5 L of the fertilizer up until they reached 50 cm in height, and twice a week thereafter. A total of 14 plants were sampled for nutrient densities in leaves and seeds. For the purpose of this study, sampling protocol followed the one we used in the field nursery study (see below).

### Field Nursery

A field nursery was established near Morris, MN (45° 41' N, 95° 48' W, elevation 370 m) on the Barnes glacial soil series during the 2005 growing season. The field site was previously in corn. Planting of cuphea was done manually (14 kg seed  $\text{ha}^{-1}$ ; May 17, 2005) and fertilizer application (110, 12 and 30 kg  $\text{ha}^{-1}$  of N, P, and K, respectively) was done mechanically at a depth of about 15 mm. The nursery was composed of six rows (6 m long and 60 cm row spacing). Weed control was carried out manually to ensure a weed-free stand.

### Soil Description

The Barnes glacial soil series was identified as the major soil type in the experimental site (Soil Survey Staff, 2004). The soil is a fine-loamy, mixed, superactive, frigid Calcic Hapludolls; it is considered as a clay loam with a plane slope of  $\sim 1\%$  on a ground of moraine. It has a calcareous subsoil horizon and typically is neutral to slightly alkaline in the surface 20 cm. This soil type

has 35.8% sand, 36.3% silt, and 27.9% clay, with a pH of 7.76. Additionally, the macro- and micronutrient contents ( $\mu\text{g g}^{-1}$ ) of this soil were determined as follows: barium (Ba; 0.046), calcium (Ca; 146.1), copper (Cu; 0.52), iron (Fe; 0.043), magnesium (Mg; 35.3), manganese (Mn; 0.73), phosphorus (P; 2.39), potassium (K; 24.2), selenium (Se; 0.001), strontium (Sr; 0.148), sulfur (S; 1.93), and zinc (Zn; 0.62).

### Leaf and Seed Sampling

Fourteen plants were sampled from the growth chamber study and fifty plants were selected at random throughout the field nursery and tagged for subsequent sampling. Four-five fully expanded green leaves per plant were sampled from the distal half of each plant at each of seven and five sampling dates in the growth chamber and field nursery studies, respectively (Table 1). Leaves from each plant, sampling date, and study were pooled to form a single sample. At physiological maturity, seeds (approximately 0.5 g per plant) were sampled with the last leaf sample. Leaf and seed samples were dried at 45°C to constant weight then used for nutrient analysis. Seed samples were separated into embryo (with cotyledons being absorbed by the embryo upon maturity) and seed coat, and nutrients were determined on each part separately.

### Determination of Macro- and Micronutrients

Plant samples (leaves and seed) were dried at 45°C in a forced air oven for a one week period or until no further reduction in weight occurred. Seed materials were ground in a coffee grinder and placed through a 1 mm screen while all other tissues were ground through a 40 mesh stainless steel Wiley mini-mill (Thomas Scientific, Swedesboro, NJ, USA). Nutrients were determined in five leaf and one seed samples from each of the growth chamber and field nursery studies separately.

Digestion of plant materials followed the US-EPA 5051 method; this procedure was adapted using the Mars Xpress Microwave System from CEM (CEM Corporation, Mathews, NC, USA) sample preparation note XprAG-1. This microwave procedure uses 55 ml Teflon tubes in a 40 unit carousel. A 0.5 g sample weight was digested with 6.5 ml nitric acid (70% Trace Metal Analysis, TMA) using a 15 min ramp program set to a power maximum of 1200 W and held for 15 min. The samples were allowed to cool to room temperature and transferred to 50 ml volumetric flasks and taken to volume with Milli-Q water (Millipore Corporation, Billerica, MA, USA). Smaller samples were taken to 25 mL with adjustments made for nitric acid ( $\text{HNO}_3$ ) concentrations. Analysis was completed using the Varian Vista-Pro CCD (Charge Coupled Device, Varian Incorporated, Palo Alto, CA, USA) simultaneous inductively coupled

Table 1

Nutrient density ( $\mu\text{g g}^{-1}$  dry weight) in five leaf samples and in mature seeds of cuphea grown in growth chamber and field nursery

Nutrient	Date (Thermal time, GDD in °C)						[S]/[L] Ratio <sup>‡</sup>
	Mean (μg g <sup>−1</sup> dry weight)						
	L1 (615) <sup>†</sup>	L2 (775)	L3 (945)	L4 (1050)	L5 (1200)	Seed (1200)	
Barium (Ba)	43.7b <sup>§</sup>	42.8b	48.6a	40.2b	50.8a	4.16 <sup>¶</sup>	0.09
	55.4b	52.8b	56.2a	57.8a	58.9a	6.9	0.12
Boron (B)	28.3a	24.7b	25.4b	20.7c	20.5c	13.3	0.56
	36.9a	29.1b	30.2b	22.1c	18.7c	15.6	0.57
Calcium (Ca)	14735d	16369c	19254a	16621c	18818b	2303	0.13
	13852c	15369b	19230a	14484c	16469b	2569	0.16
Copper (Cu)	8.1a	7.4a	9.6a	6.2ab	5.2b	14.4	1.97
	10.2a	8.4a	9.3a	7.1b	6.0b	16.5	1.98
Iron (Fe)	588.2a	380.3bc	421.0b	319.0cd	307.6d	55.6	0.14
	438.0a	392.0b	294.0c	266.0c	276.0c	59.2	0.18
Magnesium (Mg)	7704b	7547b	8222a	8545a	9019a	2788	0.34
	8919b	9273b	10446ab	11236a	11552a	3155	0.31
Manganese (Mn)	64.5a	52.6c	56.8b	49.7c	49.9c	9.91	0.19
	81.7a	73.6b	63.6c	55.2d	49.0d	10.27	0.16
Phosphorus (P)	2886a	2677b	2388c	2375c	2035d	4484	1.82
	3840a	2886b	2677b	2505b	2150c	4232	1.51
Potassium (K)	22214a	15889b	12942c	9673d	6149e	17115	1.28
	19453a	15568b	13406c	10590d	8232e	17182	1.28
Selenium (Se)	0.559a	0.428c	0.547a	0.459b	0.475b	0.019	0.040
	0.634a	0.489b	0.464b	0.379c	0.382c	0.021	0.045
Sodium (Na)	74.8d	122.1b	105.8c	142.2a	117.9b	31.5	0.28
	112.0d	123.0c	163.0a	166.0a	147.0b	43.7	0.31
Strontium (Sr)	17.1b	18.0b	20.3a	17.0b	21.7a	1.64	0.08
	22.4a	22.3a	23.2a	24.6a	25.4a	2.07	0.09
Sulfur (S)	2684a	2883a	2114b	2121b	1734c	2500	1.08
	2695a	2563a	2257b	2183b	1822c	2630	1.14
Zinc (Zn)	12.95c	12.92c	17.02b	20.45a	8.96d	33.1	2.30
	14.60c	17.80b	19.40a	19.50a	10.5d	30.8	1.90

<sup>†</sup>Leaf (L1-L5) and seed sampling dates expressed in growing degree days (GDD in  $^{\circ}\text{C}$ ).

<sup>‡</sup>Ratio of nutrient density in seed [S] and mean nutrient density in leaf [L].

<sup>§</sup>Upper and lower rows within each nutrient are nutrient densities in leaves and seed of field and growth chamber studies, respectively; Means, within each row, followed by the same letter do not differ significantly ( $p < 0.05$ , Tukey's HSD).

<sup>¶</sup>Individual nutrients in seed and leaf (L5) differed significantly from each other ( $p < 0.05$ ) using a t-test.

Plasma-optical emission spectroscopy (ICP-OES) instrument (Ryan, 2006). MNUSDA-STD 1-A and MNUSDA-STD 2 (Inorganic Ventures, Lakewood, NJ, USA) were prepared as elemental standards.

### Statistical Analyses

Descriptive statistics (mean, variance, coefficient of variation) were estimated for each nutrient in leaves and seeds of cuphea at each sampling date and growing degree days were calculated with a base temperature of 10°C. Then a density ratio ( $\mu\text{g g}^{-1}$  dry matter) for each nutrient in seed samples [S] and successive leaf samples [L] was calculated as [S]/[L] to quantify the temporal nutrient densities and nutrient variation. Shapiro-Wilk's test was conducted to test for normality of the distribution of each variable and to satisfy univariate and multivariate analysis of variance assumptions. Nutrient densities and growing degree days were log-transformed for statistical analyses (Zar, 1996; StatSoft Inc., 2007a).

Analyses of variance and mean separation (Tukey HSD, 0.05; Zar, 1996) were performed on data derived from the growth chamber and field nursery studies separately to detect significant differences among nutrient densities of leaf sampling dates (L1 to L5) and among the last leaf sampling date [L5] and seed samples at maturity [S]. The assumptions of the homogeneity of variance/covariance test were satisfied using a general linear model (StatSoft Inc., 2007b) statistical test before data from both studies were combined to perform other multivariate statistical analyses. Correlation analyses among nutrient densities were performed at three different levels: i) among nutrient densities of successive leaf samples and seeds using canonical correlation, ii) among nutrient densities in leaf samples and those in seed using product moment correlation, and iii) among nutrient densities in seed after a principal components analysis was performed to group nutrients based on their contribution to the first two principal components.

Canonical discriminant analysis was used to determine which nutrient densities could distinctly discriminate between successive leaf samples and between samples of leaves and seeds, then Mahalanobis distances ( $D^2$ ) were calculated between their centroids and tested for statistical significance. A step-wise clustering procedure (StatSoft Inc., 2007b) separated the seed produced into three significantly different classes (i.e., small, medium and large, with average seed weight of 2.4, 3.1 and 3.5 mg, respectively). The variability in nutrient densities was investigated by the use of variance components modeling; percent variance accounted for by differences between embryos and seed coats, and between seed weight classes was quantified and its level of significance was determined using general linear mixed models, GLM (StatSoft Inc., 2007a).

The temporal relationships between  $[S]/[L]$  and the unique cumulative variance for each nutrient were developed to test whether a unique rate-limiting process exists in cuphea. Regression models were developed to quantify the relationships between these two statistical characteristics of temporal nutrient densities and to identify the level of variance shared among nutrients during the vegetative and reproductive growth stages. Unique cumulative variances were derived from nutrient communalities in a factor analysis carried out at each sampling date (StatSoft Inc., 2007a). Eight critical ratios among nutrients in leaves at L1 to L5 and in seed, embryo and seed coat, along with a discrimination ratio at maturity, were calculated according to Moroni et al. (2003). These ratios were compared with published ratios for a number of oilseed (Moroni et al., 2003) and legume (Moraghan et al., 2006) crops. Statistical calculations and analyses were performed using relevant modules in the STATISTICA software package (StatSoft Inc., 2007a).

## RESULTS AND DISCUSSION

### Temporal Nutrient Densities

For each nutrient and each experiment (i.e., growth chamber and field nursery), there were significant differences between most sampling dates as indicated by the mean separation test (Tukey's HSD). However, nutrient densities were larger in leaves and seed of cuphea grown in the growth chamber as compared to those grown in the field. These differences are usually caused by differences in nutrient densities in the different growth environments (Anglani, 1998). In addition, nutrient densities in the seed differed from their corresponding values in the last leaf sampling (L5) using a *t*-test (data not presented). Temporal dynamics of nutrient densities in leaves and mature seeds of cuphea and the  $[S]/[L]$  ratio of each nutrient separated the nutrients into two groups (Table 1). The first group (Ba, Mg, Sr) with increasing  $[L]$  over time; however, these were not re-mobilized or accumulated in the seed as indicated by their small  $[S]/[L]$  ratios. The second group with decreasing  $[L]$ , whether re-mobilized and accumulated in the seed (Cu, K, P, S, Zn), or predominantly stored in leaves (B, Fe, Mn, Se). Sodium (Na) did not show a clear increasing or decreasing trend and it was largely excluded from the seed.

Deposition of nutrients in cuphea seed, typical of many plant species, (Grusak et al., 1999) is a complex process with wide differences seen in partitioning of different nutrients between leaves and seeds at the species (Tyler and Zohlan, 1998; Grusak et al., 1999) and genotypic (Feil et al., 2005; Moraghan et al., 2006) levels. Zinc appeared to be the most mobile of all nutrients, followed in decreasing order by Cu, P, K, and S (Table 1). Mobilization of Zn, which differed from the other nutrients, is closely associated with leaf senescence as suggested by Grusak et al. (1999).

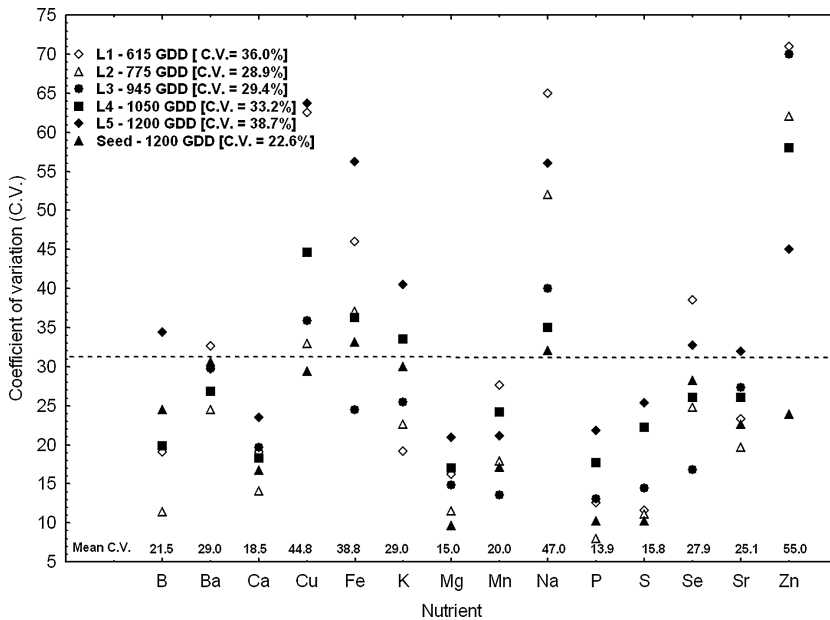
Sulfur was singled out (Naeve and Shibles, 2005) as a critical element for the reproductive development in soybean as an oilseed crop. Unlike its accumulation pattern in soybean leaves, cuphea leaves continued to re-mobilize S to the developing seed throughout the sampling period (Table 1), presumably due to the indeterminate growth habit and the continued production of flowers and seeds (Sale and Campbell, 1980, Grusak et al., 1999). Other nutrients exhibited moderate (B, Fe, and Mg) to small (Ba, Ca, Se, and Sr) mobilization rates in line with earlier findings on wheat (Grusak et al., 1999), field bean (Moraghan and Grafton, 1997), and soybean (Naeve and Shibles, 2005).

Differences among nutrients in rate and timing of mobilization are reflected on their [S]/[L] ratios (Table 1). These ranged from extremely small (0.04 for Se) to extremely large (2.3 for Zn). When compared with other plant species, some [S]/[L] ratios are unique to cuphea. For example, Tyler and Zohlan (1998) reported extremely low "seed/leaf enrichment ratio" which is equivalent to our [S]/[L] ratio, for K (0.36) and Zn (0.49) in 35 diverse plant species as compared to the respective values of 1.28 and 2.3 in cuphea. On the other hand, cuphea expressed exceptionally smaller Fe [S]/[L] ratio (0.14) as compared to 1.08 for the same 35 plant species. A small portion (18%) of Fe in cuphea L5 leaves was mobilized to the seed as compared to 20% in wheat (Calderini and Ortez-Monasterio, 2003), and ~40% in soybean (Grusak et al., 1999). Additionally, we compared [S]/[L] ratios derived from data presented by Jackson (2000) for P (2.7), K (0.59) and S (0.61) on the oilseed crop canola, with the respective values of 1.28, 2.7 and 1.08 for cuphea. Jackson (2000) also emphasized the fact that soybean leaf-[S] declined during seed filling. The same trend was found in cuphea for S, Cu, K, P, and Zn, all of which had [S]/[L] ratios > 1.0. Leaves have been reported (Naeve and Shibles, 2005) to act as a major S storage and remobilization to soybean seed for protein synthesis. Based on our data (Table 1) we speculate that it performs the same function in cuphea.

### Temporal Nutrient Variation

The wide range of variation, expressed as C.V. in leaves and seed of cuphea (Figure 1) reflects the extensive variability and the temporal dynamics among and within nutrients in cuphea, whether compared with wild plants (Tyler and Zohlan, 1998; Vreugdenhil et al., 2004) or cultivated crops (Rahamatalla et al., 1998; Moraghan et al., 2002; 2006). Variation expressed by different nutrients in cuphea seed averaged 22.6%, with Ca, Mg, Mn, P, and S having C.V. values below average; B, Ba, Fe, K, Na, and Se above average; and Sr and Zn almost average. In comparison, we calculated a mean C.V. value of 9.2% from data presented by Vreugdenhil et al. (2004) on Ca, Fe, K, Mg, Mn, P, and Zn content in seed of 25 *Arabidopsis thaliana* accessions with small values for Ca (3.6%) and Mg (3.5%) and large values for Fe (18.0%), and Mn (28.4%). Similarly, cuphea expressed larger levels of nutrient variation in the seed as compared to





**Figure 1.** Coefficients of variation (%) for macro- and micro-nutrients in five leaf samples (L1–L5) and mature seeds of cuphea.

12 genotypes of *Phaseolus vulgaris* (Fe, 11.0%, Mn, 12.0%; Moraghan et al., 2002), 12 genotypes of *Glycine max* (Ca, 3.0% and Mg, 7.0%; Moraghan et al., 2006) and four *Carthamus tinctorius* cultivars (Rahamatalla et al., 1998) with extremely small C.V. values for Cu (4–5%), Fe (1–3%), Mg (1–5%), Mn (3–5%), P (<1.0%) and Zn (3–4%). During the vegetative stage, certain nutrients (e.g., Cu, Na, and Zn) were more variable than others (e.g., Ca, Mg and P), presumably due to either the timing or their lack of mobility from vegetative tissue to the developing seed (Moraghan and Grafton, 1997).

### Correlation Among Nutrient Densities

The step-wise canonical correlation analyses among nutrients in successive leaf samples (Table 2) indicate that there were large and highly significant canonical correlations among nutrient densities throughout the growing season. However, the magnitude of the canonical r-values was time-dependent and decreased as the time lag between sampling dates increased. Canonical r-values between nutrient densities in leaves (L1 to L5) and in seed were also large and highly significant; however, the strength of these correlations increased as the sampling date approached maturity. These results suggest that the uptake,

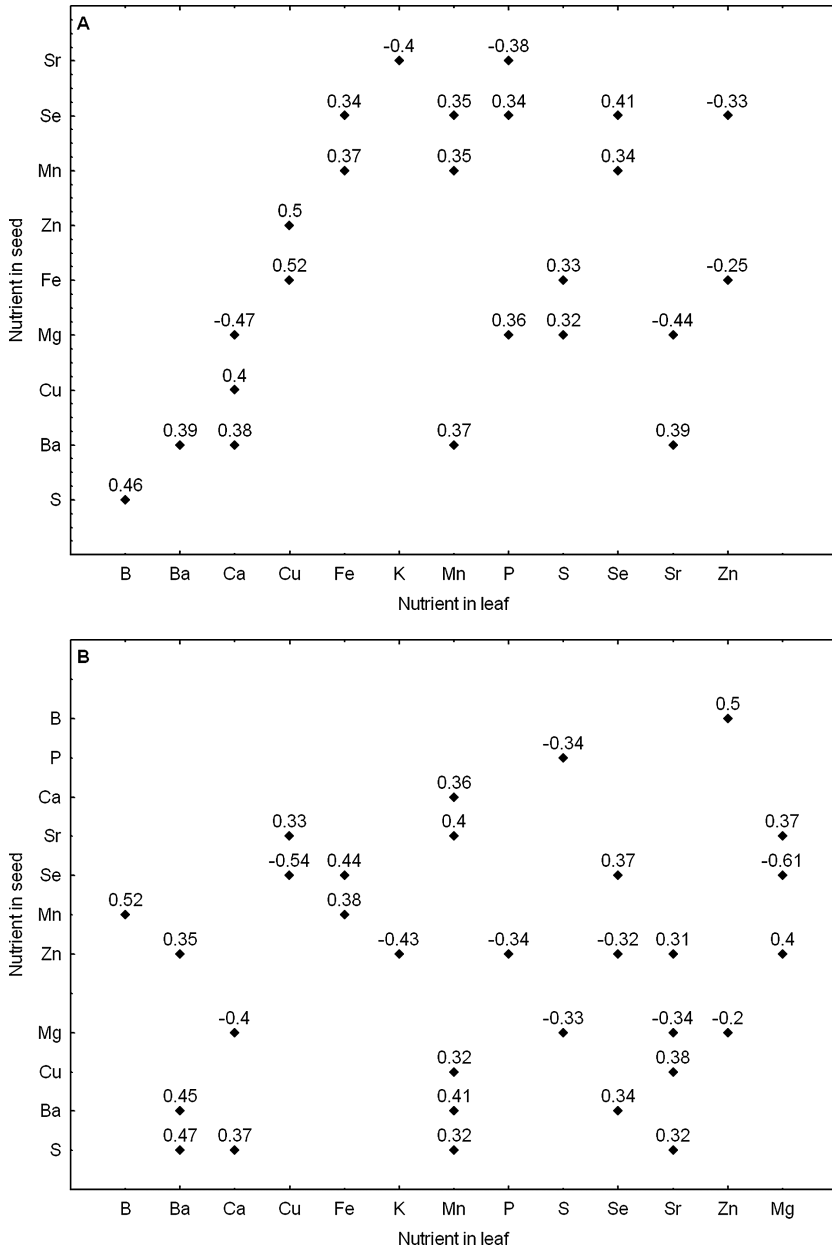
Table 2

Canonical correlations and test statistics for macro- and micronutrients in five temporal leaf and in mature seed samples of cuphea grown in a growth chamber and field nursery

Leaf sampling date (L1–L5)	Leaf/seed sampling date	Canonical R <sup>2</sup>	Chi-square	P
L1	L2	0.91	359.0	0.001
L1	L3	0.87	305.8	0.001
L1	L4	0.83	262.9	0.001
L1	L5	0.85	202.7	0.002
L1	S	0.93	207.5	0.001
L2	L3	0.94	411.6	0.001
L2	L4	0.84	256.3	0.001
L2	L5	0.83	223.0	0.00
L2	S	0.85	219.1	0.005
L3	L4	0.88	272.5	0.001
L3	L5	0.78	181.8	0.230
L3	S	0.88	222.4	0.003
L4	L5	0.80	225.0	0.002
L4	S	0.87	183.4	0.210
L5	S	0.94	234.0	0.001

storage, or remobilization to the seed of group(s) of nutrients may be mediated by physiological coupling (Ernst et al., 2000), or due to linkage of different genes (Vreugdenhil et al., 2004). Most correlation coefficients among nutrient densities in leaves and seed (Figure 2) and among nutrient densities in seed (Figure 3) were within the range of 0.3 to 0.5. This level of association may reflect the presence of flexible and highly homeostatic mechanisms in cuphea in support of nutrient uptake, accumulation, remobilization, and storage. Also, this may suggest that the rate of release of nutrients from the vegetative parts, the rate of transport from roots, or both, vary between nutrients (Sale and Campbell, 1980).

A negative impact was reported (Ali et al., 2002) for Cu on densities of Mn, N, P, and K, but not Fe or Zn in corn. In cuphea, we found a positive but not significant correlation between Fe and Cu. This relationship may indicate that [Fe] was increased in response to Cu “toxicity” (Ali et al., 2002) which may have reduced [Mn] in cuphea embryos and resulted in a significant negative correlation between these two nutrients. In cuphea, there was no evidence of the K-Mn antagonism reported by Moraghan and Grafton (2001) in common bean. These two nutrients were significantly correlated in the seed ( $r = 0.51$ ,  $p < 0.05$ ) with 49 and 59% of Mn and K stored in seed coat, respectively.



**Figure 2.** (A–E) Significant ( $p < 0.05$ ) correlation coefficients between nutrient densities in five leaf samples (L1 to L5, x-axes) and mature seed sample (y-axes) of cuphea. (Continued)

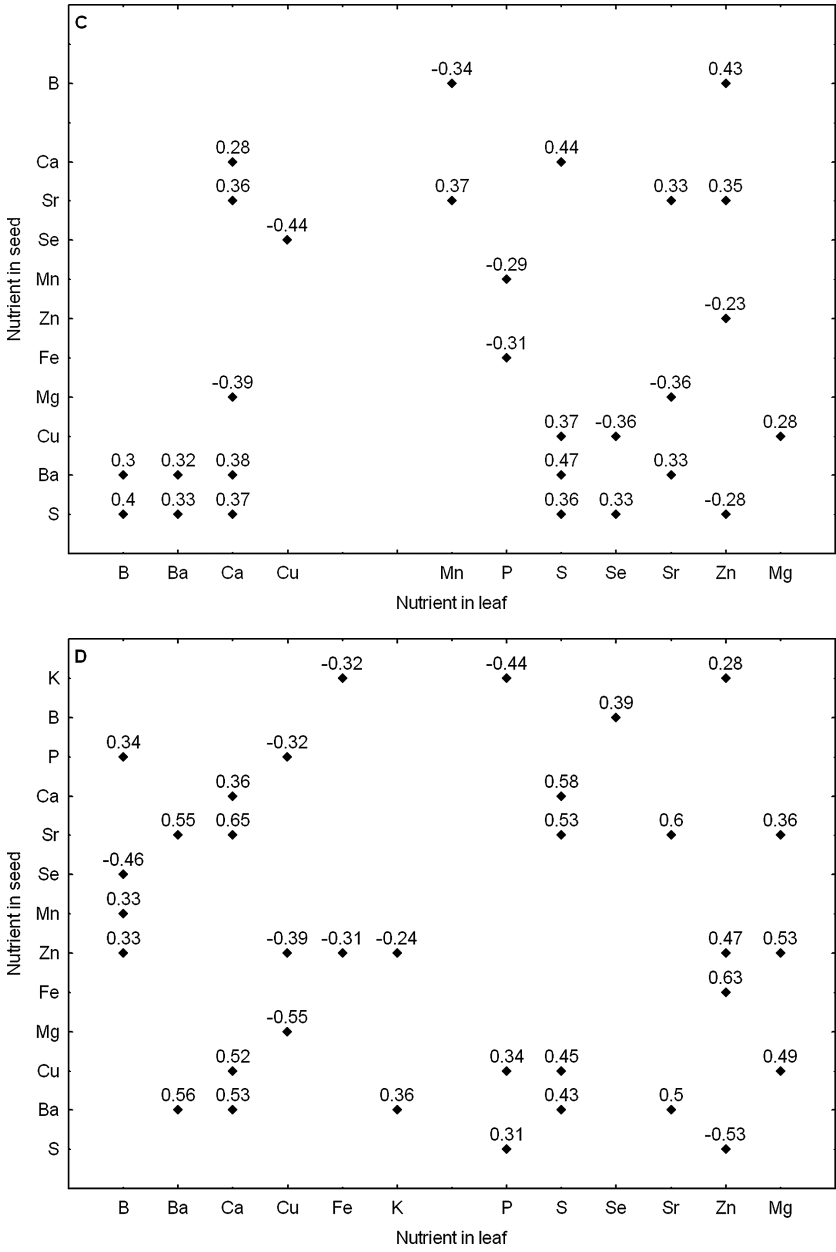


Figure 2. (Continued)

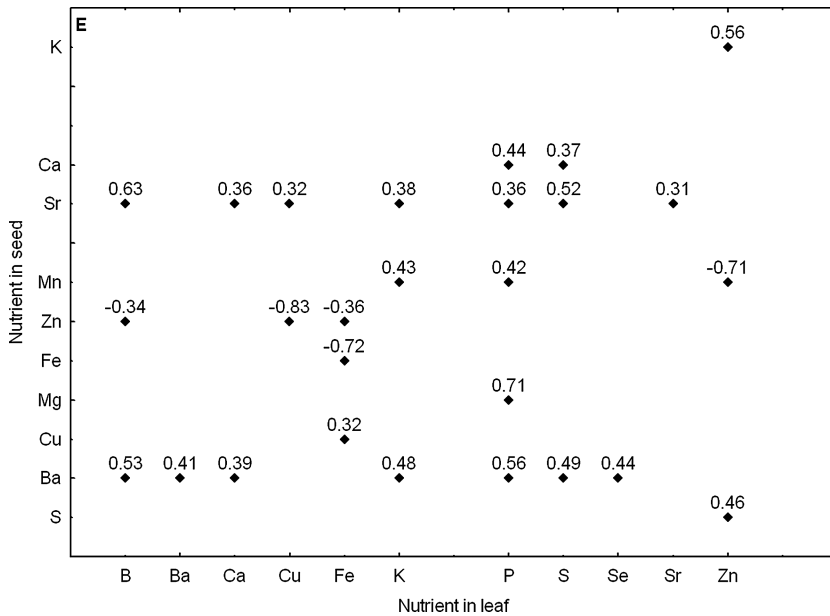
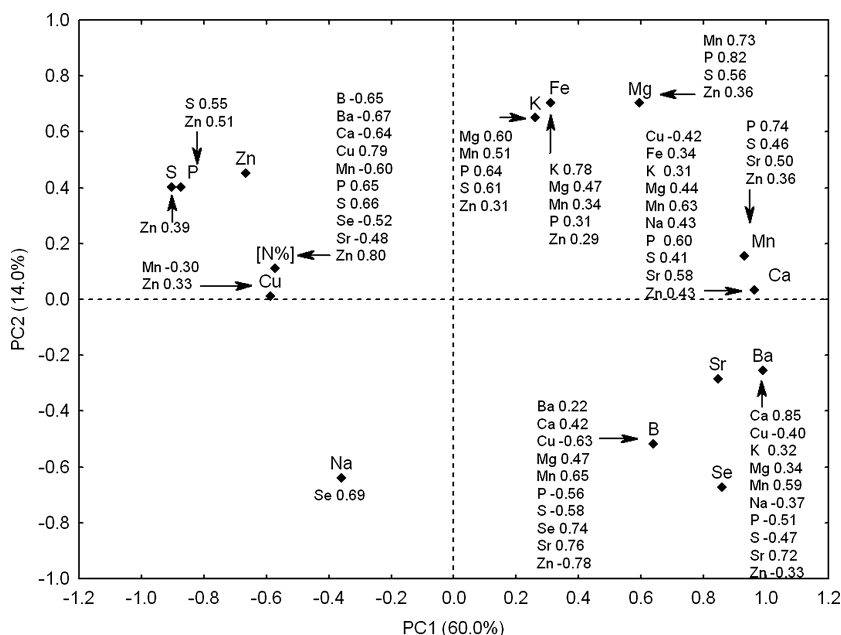


Figure 2. (Continued)

### Discrimination Among Sampling Dates

Variation among densities of 12 nutrients in leaves and seed of cuphea contributed to full (100%) discrimination among leaf and seed samples, and to a time-dependent discrimination among leaf samples with decreasing (98–70%) level of correct classification. The first two canonical discriminant roots explained 96.0% of total variance (Figure 4). The first canonical root is a linear combination, and is dominated by large loadings of Ca, Fe, P, Se, and Sr, and was orthogonal to the second root with large loadings of Ba, Cu, Mn, S and Zn. All nutrients in this group except P had small [S]/[L] ratios (Table 1). The decreasing discriminating power of the second root is reflected on the decreasing percent correct classification of the five leaf samples and in the decreasing  $D^2$  values as the sampling date approached maturity. The  $D^2$  values showed a gradual increase as the time lag between leaf sampling dates increased (e.g., 5.9 between L1 and L2 to 35.4 between L1 and L5). A similar trend was observed for  $D^2$  between seeds and leaves, albeit with much larger  $D^2$  values (range from 92.3 to 162.0).

The second discriminant root largely discriminated among the first three and last two leaf samples (i.e., at GDD of 945°C). This can be partially explained on the basis of a nutrient reaching its largest density, then declining thereafter (e.g., Ba, Cu, and Mn), or sharply decreasing towards the end of the growing

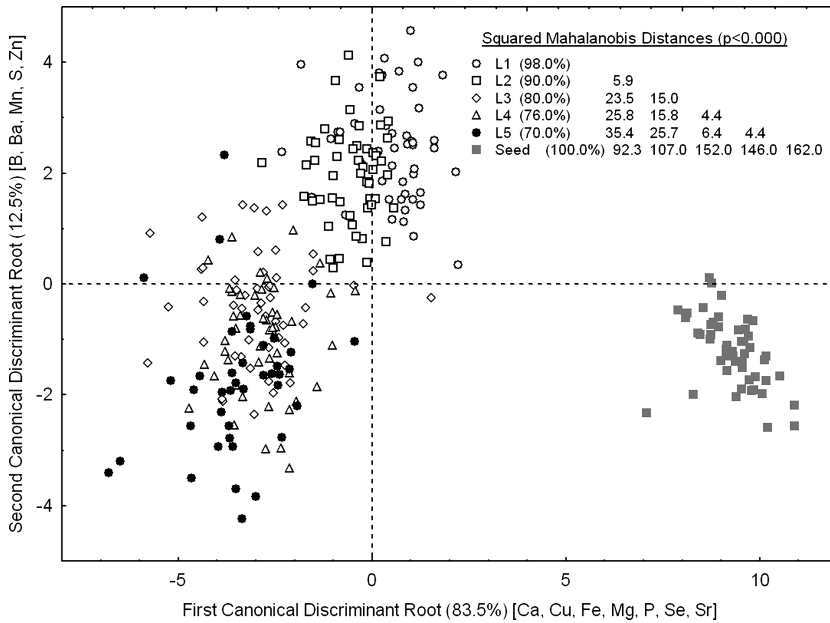


**Figure 3.** Two dimensional plot and variance explained by the first two principal components, and significant correlation coefficients between 14 macro- and micro-nutrients and N percent in mature seed samples of cuphea.

season (S and Zn). The magnitude of shifting in nutrient density tended to slow down over time and is reflected by the decreasing percent correct classification from 98.0% (L1) to 70.0% (L5) and on the small  $D^2$  values between successive sampling dates (i.e., L1–L2 of 5.9, L2–L3 of 15.0, L3–L4 of 4.4 and L4–L5 of 4.4).

### Sources of Variation in Nutrient Densities

Differences among seed coat and embryo accounted for small (7.6%) and large (84.5%) portions of variation in Mn and K, respectively; whereas, differences among seed weight classes explained significant portions of variation in 10 nutrients (Table 3). Nutrient densities differed significantly among embryo and seed coat with embryo accumulating more Cu, Fe, Mg, P, S, and Zn than the seed coat (Table 3). The remaining nutrients were larger in seed coat; whereas, Mn was accumulated in embryo and seed coat in almost equal densities. Large Ca (156 fold), K (1.46 fold), Na (2.8 fold) and Sr (20.5 fold) densities were found in seed coat as compared to their respective densities in the embryo;



**Figure 4.** Percent correct classification and squared Mahalanobis distances ( $D^2$ ,  $p < 0.001$ ) among centroids of five leaf and one mature seed samples based on the first two canonical discriminant roots of macro- and micro-nutrients of cuphea.

whereas, embryos had larger densities of Mg (3.2 fold), P (10.4 fold), S (3.3 fold) and Zn (1.6 fold) than the seed coat.

Genotypic (Moraghan et al., 2002) and species' (Vreugdenhil et al., 2004) differences have been reported as to the proportion of nutrients stored in seed coat and in embryo of crops and wild plants. Large proportions (74.0–99.0%) of nutrients “deleterious” to the embryo (i.e., B, Ba, Ca, Na, Se, and Sr) were stored in the seed coat, whereas most “beneficial” nutrients (i.e., Cu, Fe, K, Mg, Mn, P, S and Zn) were stored in large proportions (41.0–92.0%) in the embryo. This is in agreement with reported results on field beans (*Phaseolus vulgaris* L.) (Moraghan and Grafton, 2001) and *Arabidopsis thaliana* (Vreugdenhil et al., 2004) and was attributed to the relative proportions of seed coat to embryo in seeds of these crops. Generally, small amounts of P are stored in the seed coat in contrast to large amounts of Ca, Mg and Zn so that the relative portions of these nutrients bound to phytate is small (Vreugdenhil et al., 2004; Moraghan et al., 2006). Cuphea stored 8% of total P, 99.0% of total Ca, 24.0% of total Mg, and 38.0% of total Zn in the seed coat compared to 25 *Arabidopsis thaliana* accessions which stored 3.0% of P and 40.0–60.0% of Ca, Mg, and Zn in the seed coat (Vreugdenhil et al., 2004), and *Phaseolus vulgaris* which stored

Table 3

Mean separation among macro- and micronutrients, and percent variance due to differences between the embryo and seed coat, and between three seed weight classes of cuphea

Nutrient	Mean (mean separation, Tukey's HSD)			Percent variance due to differences between	
	Embryo	Seed coat	Percent in seed coat	Embryo & seed coat	Seed weight classes <sup>§</sup>
Barium (Ba)	0.24b <sup>†</sup>	3.92a	80	92.0** <sup>‡</sup>	6.9*
Boron (B)	2.72b	10.58a	94	89.2**	2.5*
Calcium (Ca)	14.62b	2288.4a	99	90.9**	8.7*
Copper (Cu)	9.29a	5.11b	55	89.3**	
Iron (Fe)	31.4a	24.2b	44	37.3*	8.0*
Magnesium (Mg)	2118.2a	669.8b	24	90.3**	6.2*
Manganese (Mn)	5.03a	4.88a	49	7.6*	68.6**
Phosphorus (P)	4090.4a	393.6b	8	92.9**	5.3*
Potassium (K)	6950b	10165a	59	84.5**	
Selenium (Se)	0.0036b	0.0154a	81	67.1**	12.6*
Sodium (Na)	8.28b	23.2a	74	90.4**	
Strontium (Sr)	0.01b	0.018a	95	92.4**	7.0*
Sulfur (S)	1923.1a	576.9b	23	93.4**	6.1*
Zinc (Zn)	20.5a	12.56b	38	59.3**	

<sup>†</sup>Nutrient means in embryo and seed coat, within each row, followed by the same letter do not differ significantly ( $p < 0.05$ , Tukey's HSD).

<sup>‡</sup>\*, \*\*significant at  $p < 0.05$  and  $p < 0.01$ , respectively.

<sup>§</sup>Seed classes: Light(2.4 mg/seed), Medium(3.1 mg/seed), Heavy(3.5 mg/seed).

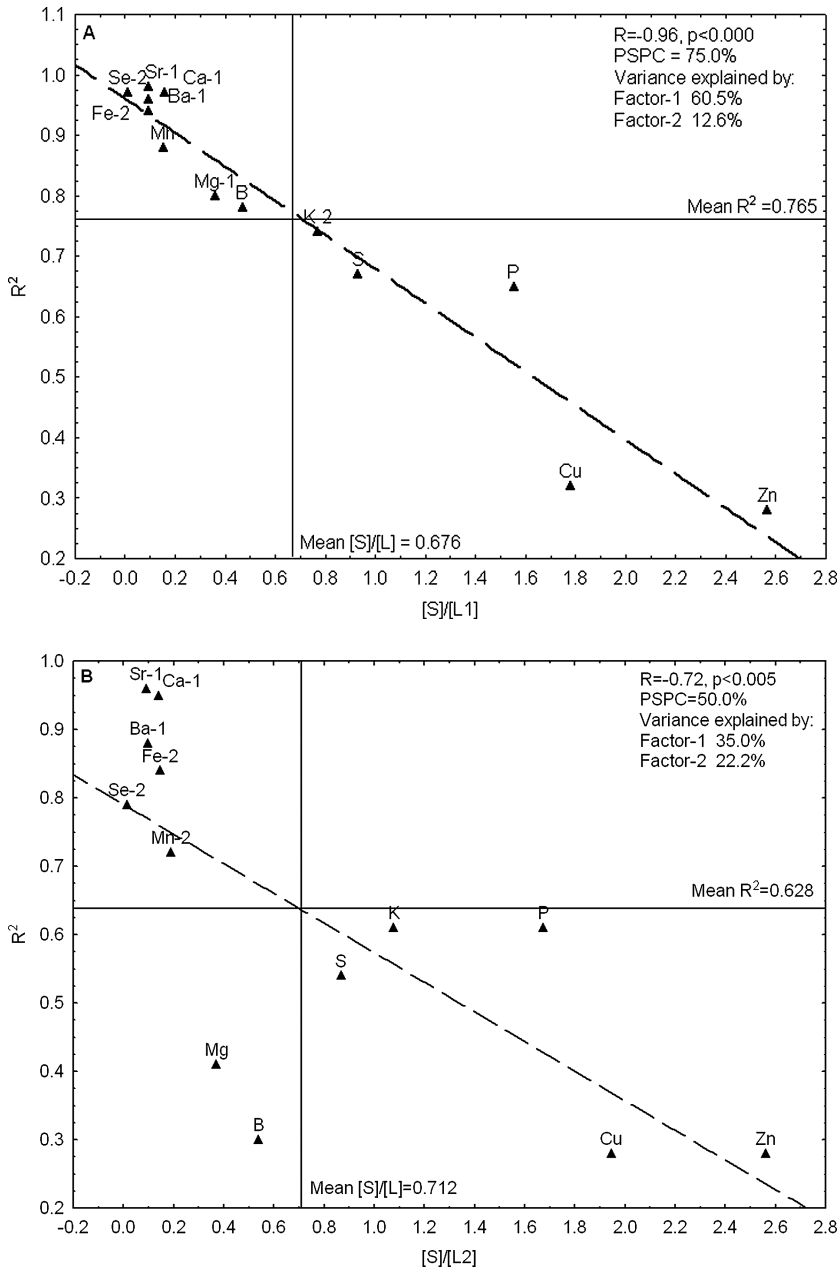
80.0% of Ca in the seed coat, and *Glycine max* which stored 25.0% of Ca in the seed coat (Moraghan et al., 2006).

Tannin content is expected to be large in the black seed coat of cuphea (Hirsinger and Knowles, 1984). It is, therefore, speculated that the large Fe density in its seed coat (44.0% of total Fe) is related to the presence of tannins which can complex Fe. The relative [Fe] in seed coat and embryo could be a limiting factor in providing necessary Fe for the germinating embryo if most of it is stored in the seed coat as it is the case in "colored" field bean (*Phaseolus vulgaris* L.) genotypes (Moraghan et al., 2002).

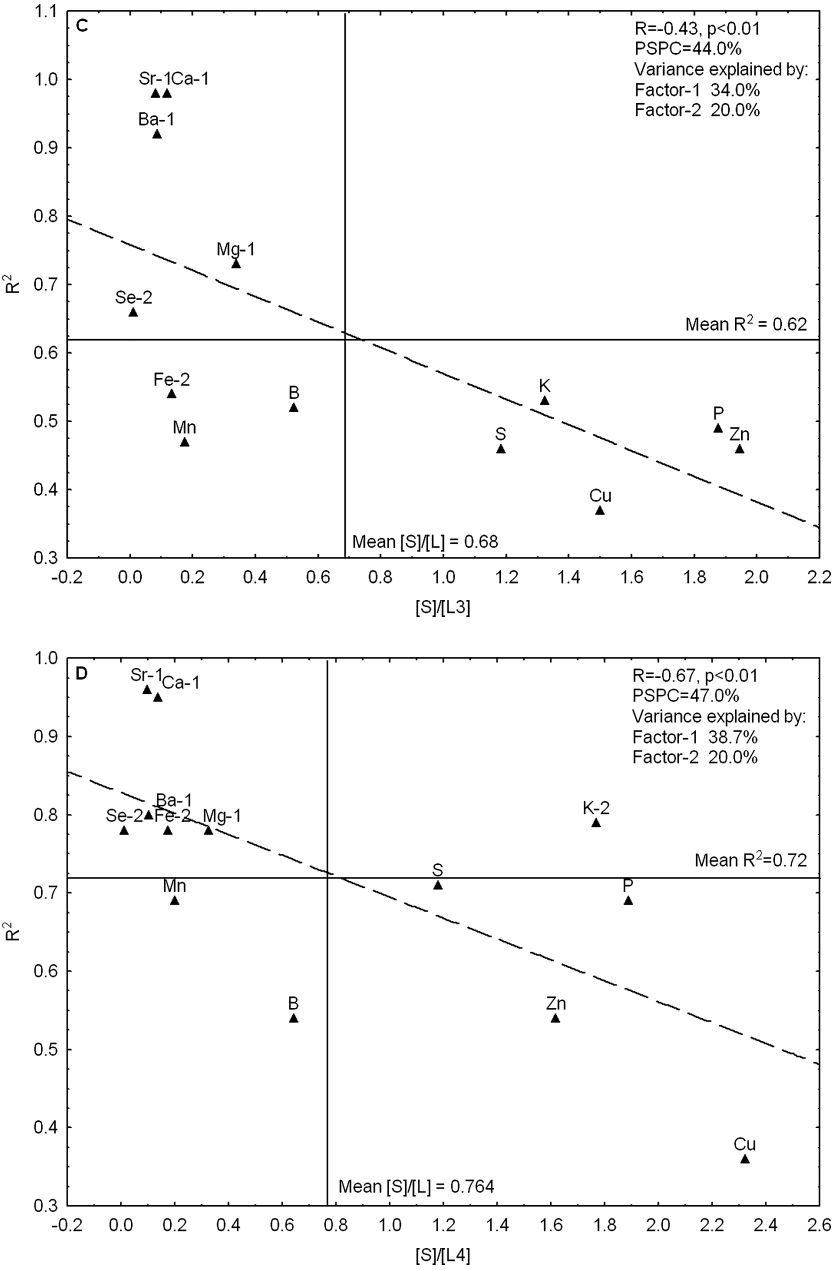
### Nutrient Dynamics

Percent significant pairwise comparisons (PSPC) among nutrient mean densities were large during the early part of the growing season, then dropped





**Figure 5.** (A–E) Relationship between seed-to-leaf ratio,  $[S]/[L]$ , and cumulative coefficient of determination ( $R^2$ ) derived from communalities on the first two factors of macro-, and micro-nutrients in five leaf (L1–L5) and one mature seed samples of cuphea. (Continued)



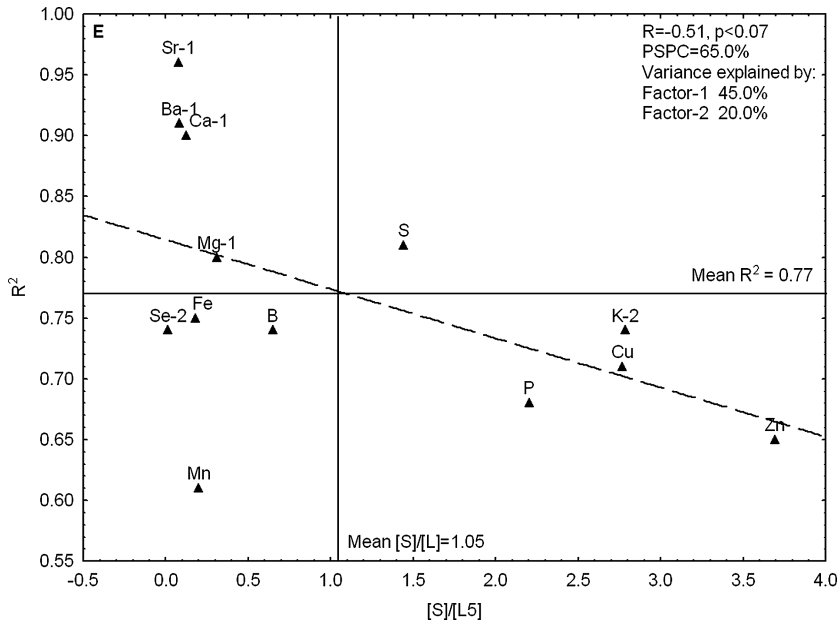


Figure 5. (Continued)

steadily from 75.0 (Figure 5, L1) to 47.0% just before maturity (Figure 5, L4) then increased to 65.0% at full maturity. Two factors accounted for 54.0 to 73.1% of total variance in nutrients of leaves sampled during the growing season. The composition of these two factors was not static during the sampling period. Barium, Ca, Mg and Sr predominantly loaded on Factor 1 while Fe, K, Mn, and Se loaded on Factor 2. Although Ba, Ca, Mg, Se, and Sr constantly loaded high on either factor, their  $R^2$  and  $[S]/[L]$  ratios were shifting within a narrow range during the sampling period, and unlike nutrients with low  $R^2$  and high  $[S]/[L]$  values such as S and Zn. The  $[S]/[L]$  ratios of Ba, Ca, Mg, Se, and Sr were smaller than mean  $[S]/[L]$  ratio in each sampling date. Correlation coefficients between  $R^2$  and  $[S]/[L]$  estimates were highly significant and showed a gradual decrease over time (i.e., from  $-0.72$ ;  $p < 0.001$ , for L1, to  $-0.51$ ,  $p < 0.07$ , for L5).

The dynamic relationship between  $[S]/[L]$  ratios and the proportion of variance that is unique to each nutrient (i.e.,  $1 - R^2$ ) classified most nutrients into two distinct groups in accordance with their storage in seed coat or embryo (Table 1), and in line with their rate of remobilization to the developing seed (Blevins and Lukaszewski, 1998; Eriksen et al., 2001). The statistical evidence (Figure 1 and Table 1) suggests that certain nutrients (e.g., Cu, K, P, S, and to some extent Zn, with large C.V. values) have been steadily remobilized to the seed more than others (Ca, with almost constant  $[S]/[L]$  ratio over time), and

both of their [S]/[L] ratios and unique variances increased over time. Three statistics in the regression equations (i.e., the intercept, slope, and *r*-value; Figure 5) point to the diminishing power of [S]/[L] ratio in determining the unique variance for certain nutrients as they are being remobilized to the seed. Values of the intercept decreased from 0.96 to 0.81, the slope from  $-0.29$  to  $-0.04$ , and *R* from 0.96 ( $p < 0.00$ ) to 0.51 ( $p < 0.07$ ). Nutrients with above mean [S]/[L] ratios and above mean unique variances (mostly Cu, S, and Zn) share very little of their variances with other nutrients included in the analysis; whereas, nutrients with large  $R^2$  values share most of their variances with other nutrients. This dynamism rules out the existence of a unique rate limiting process (Grusak et al., 1999) and confirms that wide differences exist in nutrient partitioning between vegetative and reproductive tissues in cuphea.

### Discrimination Ratios Among Nutrients

Four distinct patterns can be identified in discrimination ratios (Table 4) based on their trends between L1 and L5. The first pattern with values reaching a maximum at L3 then decreasing (Cu/Fe and Cu/Mn), or not (Ca/Mg); the second with a steady increasing (Mn/Fe) or decreasing (Mn/Mg) ratio; the third with values reaching a maximum at L4 and decreasing at L5 (Zn/Fe and Zn/Mn); and the fourth (Se/S) with no clear trend. When critical ratios were expressed on embryo and seed coat basis, the Cu/Mn and Zn/Mn ratios were  $>1.0$  in both embryo and seed coat, and Ca/Mg was  $>1.0$  only in seed coat. Large (Ca/Mg), medium (Cu/Mn and Zn/Mn), and small (Cu/Fe, Mn/Fe, and Zn/Fe) differences were observed between embryo and seed coat critical ratios. Finally, discrimination ratios at seed maturity (i.e., between L5 and seed) were large ( $>14.0$ ) for most nutrients except for Ca/Mg (0.4), and were negligible for Se/S.

Discrimination ratios can be viewed within the context of bi-variate correlations among nutrients (Moroni et al., 2003). For example, Ca and Mg, were negatively and significantly correlated at L1 ( $r = -0.47$ ,  $p < 0.05$ ), L2 ( $r = -0.40$ ,  $p < 0.05$ ), and L3 ( $r = -0.39$ ,  $p < 0.05$ ). These *r*-values were reflected on a discrimination ratio of 0.4. On the other hand, Cu and Fe at L1 ( $r = 0.52$ ,  $p < 0.05$ ), Mn and Fe at L2 ( $r = 0.38$ ,  $p < 0.05$ ), and Zn and Fe at L5 ( $r = 0.63$ ,  $p < 0.01$ ) were positively and significantly correlated with discrimination ratios  $> 1.0$ . Cuphea seems to discriminate against Ca in favor of Mg; against Fe in favor of Cu, Mn, and Zn; against Mn in favor of Cu and Zn; and against Mg in favor of Mn. The discrimination ratio for Se/S (Table 4) is negligible; however, the chemical and physical resemblance between Se and S establishes that they share common metabolic pathways in plants (Sors et al., 2005). Selenium was preferentially stored in the seed coat (81%) as compared to 23% of S (Table 3) and displayed a totally different time-dependent mobilization pattern from leaves to seed (Table 1).

Table 4  
Critical and discrimination ratios among macro- and micronutrients in five leaf (L1-L5) and one mature seed samples of cuphea

Nutrient	Sampling date					Seed part			
	L1	L2	L3	L4	L5	Seed	Discrimination ratio	Embryo	Seed coat
Ca/Mg	1.91	2.17	2.34	1.95	2.08	0.83	0.40	0.007	3.42
Cu/Fe	0.0138	0.0195	0.0228	0.0194	0.0169	0.26	15.3	0.30	0.21
Cu/Mn	0.126	0.141	0.169	0.125	0.104	1.45	14.0	1.85	1.04
Mn/Fe	0.109	0.138	0.134	0.155	0.163	0.176	1.07	0.160	0.20
Mn/Mg	0.0084	0.0069	0.0069	0.0058	0.0001	0.0036	36.0	0.0024	0.0073
Zn/Fe	0.022	0.034	0.04	0.064	0.029	0.60	20.7	0.65	0.52
Zn/Mn	0.20	0.25	0.30	0.41	0.18	3.34	18.6	4.08	2.75
Se/S	0.0002	0.00015	0.00025	0.00022	0.00027	0.0	0.0	0.0	0.0

Most studies on Fe and Mn interaction have found a negative correlation between their accumulation in the shoots of susceptible *Brassica* spp. (Moroni et al., 2003). In cuphea, we did not find evidence of Mn interference with Fe. The Mn/Mg discrimination ratio does indicate that Mn interferes with Mg as reported by Moroni et al. (2003). A positive and significant correlation was found between Fe and Mn ( $r = 0.34$ ,  $p < 0.05$ ). The [Fe] in cuphea seed was  $55.6 \mu\text{g g}^{-1}$ , which is slightly above the critical deficiency level (Grusak et al., 1999).

## CONCLUSIONS

Homeostatic mechanisms that delineate nutrient accumulation and remobilization in cuphea, their dynamics, and interrelationships were quantified as prerequisites for its development as a competitive, industrial oilseed crop. Fourteen macro- and micronutrients exhibited large variation in leaves and seeds of cuphea during the growing season, some nutrients (sulfur and zinc) were remobilized to the developing seed in large amounts, while others (barium and strontium) were almost totally excluded from the seed. We found that copper, iron, sulfur, and zinc are accumulated in the embryo, whereas barium, calcium, magnesium, manganese and strontium are largely accumulated in the seed coat. Increasing nutrients of the first group in cuphea seeds would result in more seedling vigor and viability, thus enhancing the performance of seedlings upon germination. Nutrients with large densities in leaves at harvest (e.g., Ca, Fe, P, K, and S) may constitute a resource potentially available for subsequent crops. This information would help plant breeders exploit genotypic variability in seed nutrient concentration and agronomists identify critical nutrients and plan fertilizer programs for this potential oilseed crop.

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## REFERENCES

- Ali, A. N., M. P. Bernal, and M. Ater. 2002. Tolerance and bioaccumulation of copper in *Phragmites australis* and *Zea mays*. *Plant and Soil* 239: 103–111.
- Anglani, C. 1998. Wheat minerals-A review. *Plant Foods for Human Nutrition* 52: 177–186.

- Blevins, D. G., and K. M. Lukaszewski. 1998. Boron in plant structure and function. *Annual Reviews of Plant Physiology and Plant Molecular Biology* 49: 481–500.
- Brink, G. E., G. A. Pederson, K. R. Sistani, and T. E. Fairbrother. 2001. Uptake of selected nutrients by temperate grasses and legumes. *Agronomy Journal* 93: 887–890.
- Calderini, D. F., and I. Orteza-Monasterio. 2003. Grain position affects macronutrient and micronutrient concentration in wheat. *Crop Science* 43: 141–151.
- Dierig, D. A., C. M. Grieve, and M. C. Shannon. 2003. Selection for salt tolerance in *Lesquerella fendleri* (Gray) S. Wats. *Industrial Crops and Products* 17: 15–22.
- Eriksen, J., M. Nielsen, J. V. Mortensen, and J. K. Schjørring. 2001. Redistribution of sulfur during generative growth of barley plants with different sulfur and nitrogen status. *Plant and Soil* 230: 239–246.
- Ernst, W. H. O., H. J. M. Nelissen, and W. M. Ten Brookum. 2000. Combination toxicology of metal-enriched soils: Physiological responses of a Zn- and Cd-resistant ecotypes of *Silene vulgaris* on polymetallic soils. *Environmental and Experimental Botany* 43: 55–71.
- Feil, B., S. B. Moser, S. Jampatong, and P. Stamp. 2005. Mineral composition of the grain of tropical maize varieties as affected by pre-anthesis drought and rate of nitrogen fertilization. *Crop Science* 45: 516–523.
- Grusak, M. A., J. N. Pearson, and E. Marentes. 1999. The physiology of micronutrient homeostasis in field crops. *Field Crops Research* 60: 41–56.
- Hirsinger, F., and P. F. Knowles. 1984. Morphological and agronomic descriptors of selected *cuphea* germplasm. *Economic Botany* 38: 439–451.
- Jackson, G. D. 2000. Effects of nitrogen and sulfur on canola yield and nutrient uptake. *Agronomy Journal* 92: 644–649.
- Knapp, S. J., and J. M. Crane. 2000. Registration of reduced shattering cuphea germplasm PSR23. *Crop Science* 41: 299–300.
- Moraghan, J. T., J. D. Etchevers, and J. Padilla. 2006. Contrasting accumulation of calcium in seed coats and embryos of common bean and soybean. *Food Chemistry* 95: 554–561.
- Moraghan, J. T., and K. Grafton. 1997. Accumulation of calcium in bean cultivars differing in seed size. *Journal of the Science of Food and Agriculture* 74: 251–256.
- Moraghan, J. T., and K. Grafton. 2001. Genetic diversity and mineral composition of common bean seed. *Journal of the Science of Food and Agriculture* 81: 404–408.
- Moraghan, J. T., J. Padilla, J. D. Etchevers, K. Grafton, and J. A. Acosta-Gallegos. 2002. Iron accumulation in seed of common bean. *Plant and Soil* 246: 175–183.

- Moroni, J. S., B. J. Scott, and N. Wratten. 2003. Differential tolerance of high manganese among rapeseed genotypes. *Plant and Soil* 253: 507–519.
- Naeve, S. L., and R. M. Shibles. 2005. Distribution and mobilization of sulfur during soybean reproduction. *Crop Science* 45: 2540–2551.
- Olness, A. E., D. W. Archer, R. W. Gesch, and J. Rinke. 2004. Resin-extractable phosphorus, vanadium, calcium and magnesium as factors in maize (*Zea mays* L.) yield. *Journal of Agronomy and Crop Science* 188: 94–101.
- Rahamatalla, A. B., E. E. Babiker, A. G. Krishna, and A. H. El Tinay. 1998. Changes in chemical composition, minerals and amino acids during seed growth and development of four safflower cultivars. *Plant Foods for Human Nutrition* 52: 161–170.
- Ryan, A., 2006. VARIAN-Rapid measurements of major, minor and trace elements in plant and food materials using Varian 730-ES. Available at [www.varianinc.com](http://www.varianinc.com) (Accessed September 1, 2007).
- Sale, P. W. G., and L. C. Campbell. 1980. Patterns of mineral nutrient accumulation in soybean seed. *Field Crops Research* 3: 157–163.
- Soil Survey Staff. 2004. Natural Resources Conservation Service, United States Department of Agriculture, Official Soil Series. Available at <http://soils.usda.gov/technical/classification/osd/index.html> (Accessed August 17, 2007).
- Sors, T. G., D. R. Ellis, and D. E. Salt. 2005. Selenium uptake, translocation, assimilation and metabolic fate in plants. *Photosynthesis Research* 86: 373–389.
- StatSoft Inc. 2007a. STATISTICA, Version 7.1. Tulsa, OK: StatSoft.
- StatSoft Inc. 2007b. Electronic statistics textbook. Available at: <http://www.statsoft.com/textbook/stathome.html> (Accessed September 1, 2007).
- Tyler, G., and A. Zohlan. 1998. Plant seed as mineral nutrient resource for seedlings – A comparison of plants from calcareous and silicate soils. *Annals of Botany* 81: 455–459.
- Vreugdenhil, D., M. G. M. Aarts, M. Koornneef, H. Nelissen, and W. H. O. Ernst. 2004. Natural variation and QTL analysis for cationic mineral content in seeds of *Arabidopsis thaliana*. *Plant Cell and Environment* 27: 828–839.
- Zar, J. H., 1996. *Biostatistical Analysis*, 3rd ed. Upper Saddle River, NJ: Prentice Hall.